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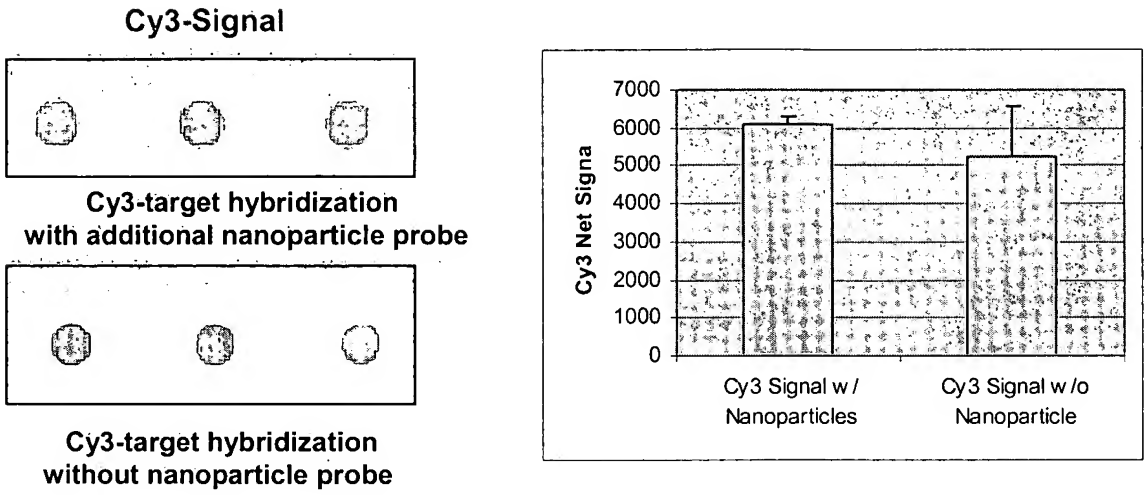
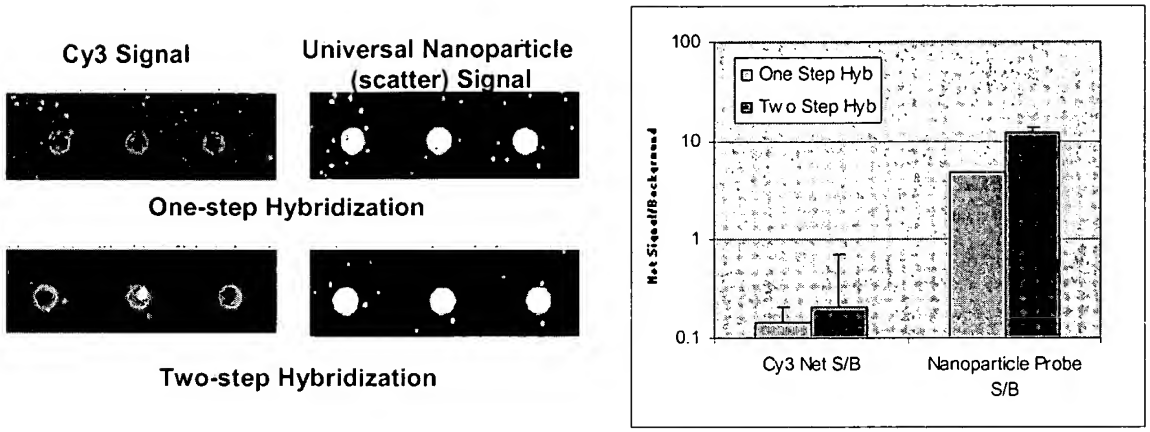
FIG. 1



Universal nanoparticle probe does not interfere with Cy3 fluorescent probe hybridization. Cy3-labled target hybridization on the test array in the presence or absence of nanoparticle poly dA-probe. Experiment conditions: Gene specific RNA targets and corresponding capture oligos were purchased from Ambion (sequence information is not available). Capture oligos (30uM) were spotted on CodeLink slides. Cy3-labeled cDNA targets were hybridized on microarray with or without oligo-dA 20mer gold particle probe in a mixture containing at 30% formamide, 5XSSC, and 0.05% TWEEN 20 at 40oC for 1 hour. After hybridization, the array was washed in 0.5M NaNO3 and 0.05% TWEEN 20 at room temperature for 2 min (2X), in 2XSSC at room temperature for 2 min, and then in 0.5XSSC for 0 seconds. After spin dry, the slide was imaged with Arraywork at Cy3 channel.

FIG. 2



Cy3 signal and the nanoparticle (scatter) signal on the array after hybridization with target and nanoparticle probe. Experiment conditions: Gene specific RNA targets and corresponding capture oligos were purchased from Ambion (sequence information is not available). Capture oligos (30uM) were spotted on CodeLink slides. In a one-step hybridization, Cy3-labeled cDNA targets and oligo-dA 20mer gold particle probe were co-hybridized on microarray in a mixture containing 30% formamide, 5XSSC, and 0.05% TWEEN 20 at 40oC for 1 hour. After hybridization, the array was washed in 0.5M NaNO3 and 0.05% TWEEN 20 at room temperature for 2 min (2X), in 2XSSC at room temperature for 2 min, and then in 0.5XSSC for 10 seconds. After spin dry, the slide was imaged with Arraywork at Cy3 channel. The slide was further washed with 0.5M NaNO3, and then subjected to silver stain to obtain scatter signal. In a two-step hybridization, Cy3-labeled cDNA targets were hybridized on microarray in a mixture containing 30% formamide, 5XSSC, and 0.05% TWEEN 20 at 40oC for 1 hour. After hybridization, the array was washed in 0.5M NaNO3 and 0.05% TWEEN 20 at room temperature for 2 min (2X), in 2XSSC at room temperature for 2 min, and then in 0.5XSSC for 10 seconds. After spin dry, the slide was imaged with Arraywork at Cy3 channel. The slide was then hybridized with oligo dA-20mer gold particle probe in a mixture containing 30% formamide, 5XSSC, and 0.05% TWEEN 20 at 40oC for 45 min. After probe hybridization step, the slide was washed with in 0.5M NaNO3 and 0.05% TWEEN 20 at room temperature for 2 min (2X), and then the slide was subjected to silver stain to obtain scatter signal.

FIG. 3

FIG. 3a

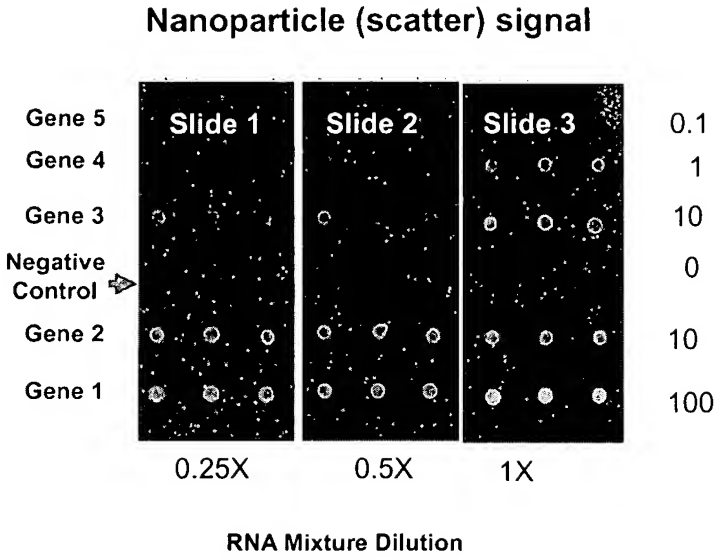
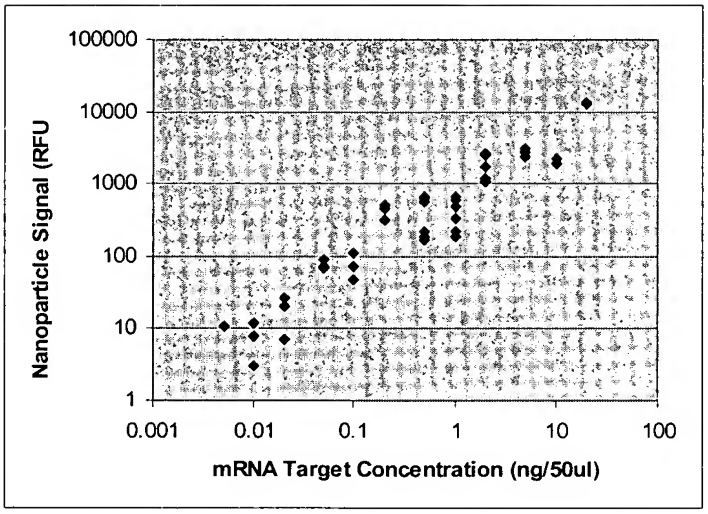


FIG. 3b



Nanoparticle (scatter) signal on test arrays in a target titration experiment. Experiment conditions: Gene specific RNA targets and corresponding capture oligos were purchased from Ambion(sequence information is not available). Capture oligos (30uM) were spotted on CodeLink slides. In a one-step hybridization, the RNAs were mixed at different concentrations as indicated in Fig. 3a and then labeled with Cy3. The Cy3-labeled cDNA targets and oligo-dA 20mer gold particle probe were co-hybridized on microarrays in a mixture containing 30% formamide, 5XSSC, and 0.05% TWEEN 20 at 40oC for 1 hour. After hybridization, the array was washed in 0.5M NaNO3 and 0.05% TWEEN 20 at room temperature for 2 min (2X), in 2XSSC at room temperature for 2 min, and then in 0.5XSSC for 10 seconds. After spin dry, the slide was imaged with Arraywork at Cy3 channel. The slide was further washed with 0.5M NaNO3, and then subjected to silver stain to obtain scatter signal.

Note: Data collected from 3 hybridizations with serial target dilutions.

FIG. 4

FIG. 4a

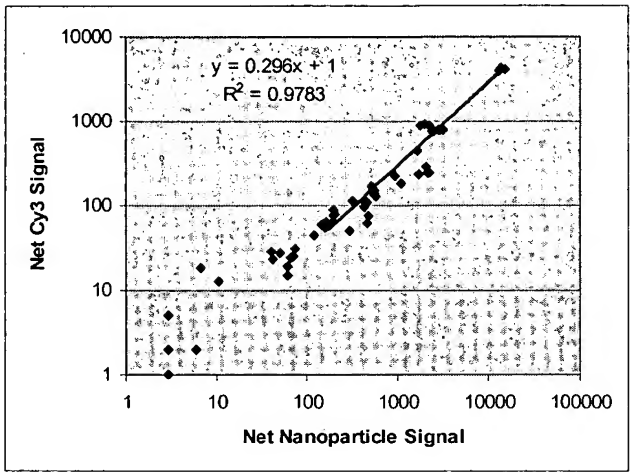
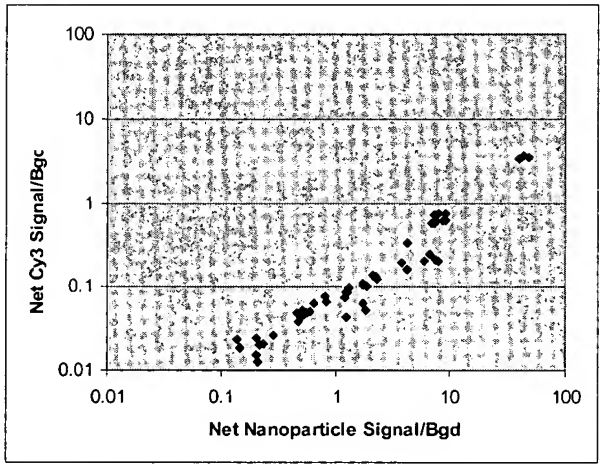
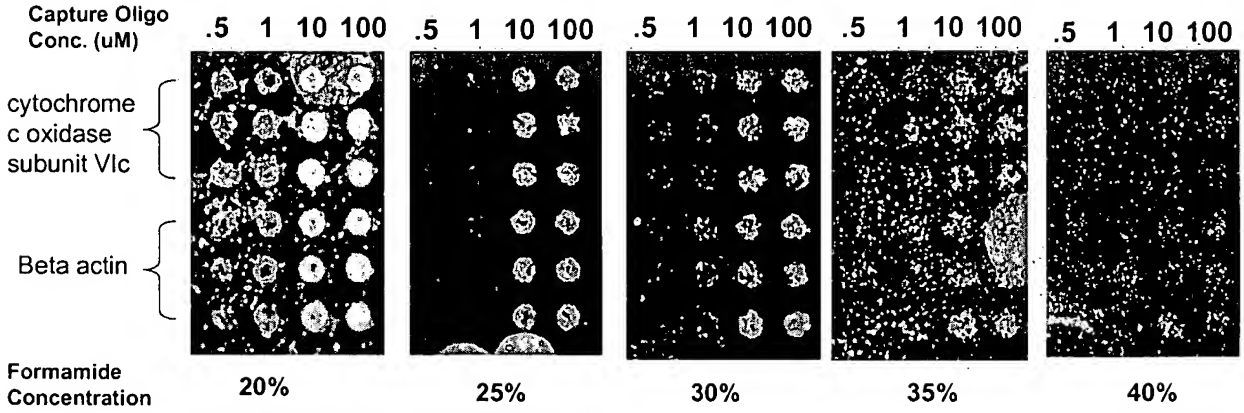


FIG. 4b



Correlation of nanoparticle (scatter) signals and Cy3-fluorescent signals. Figure 4a is Scatter plot of net nanoparticle Signals vs. net Cy3-fluorescent signals. >3 log linear correlation observed between nanoparticle signal and fluorescent signal. Figure 4b. Scatter plot of net nanoparticle signal/background ratios vs. net Cy3-fluorescent signal/background ratios. The signal/background ratio with nanoparticle probe is 10-40 fold higher than Cy3 probe at all target cocentrations. Experiment conditions: Gene specific RNA targets and corresponding capture oligos were purchased from Ambion(sequence information is not available). Capture oligos (30uM) were spotted on CodeLink slides. In a one-step hybridization, the RNAs were mixed at different concentrations as indicated in Fig. 3a and then labeled with Cy3. The Cy3-labeled cDNA targets and oligo-dA 20mer gold particle probe were co-hybridized on microarrays in a mixture containing 30% formamide, 5XSSC, and 0.05% TWEEN 20 at 40oC for 1 hour. After hybridization, the array was washed in 0.5M NaNO3 and 0.05% TWEEN 20 at room temperature for 2 min (2X), in 2XSSC at room temperature for 2 min, and then in 0.5XSSC for 10 seconds. After spin dry, the slide was imaged with Arraywork at Cy3 channel. The slide was further washed with 0.5M NaNO3, and then subjected to silver stain to obtain scatter signal.

FIG. 5



Lower capture oligo concentration to reduce probe-capture interaction. Experiment conditions: Two capture oligos (beta actin and XX) were spotted on CodeLink slides. The oligo-dT 20mer gold particle probe (1 nM) was added on to microarray in a mixture containing 20%-40% formamide, 4XSSC, and 0.04% TWEEN 20, at 40oC for 30 min. After hybridization, the arrays were washed in 0.5M NaNO3 and 0.05% TWEEN 20 at room temperature for 2 min (4X), and 0.5M NaNO3 at room temperature for 2min (2X). The slides were subjected to silver stain (5.5 min) to obtain scatter signal.

FIG. 6

FIG. 6a

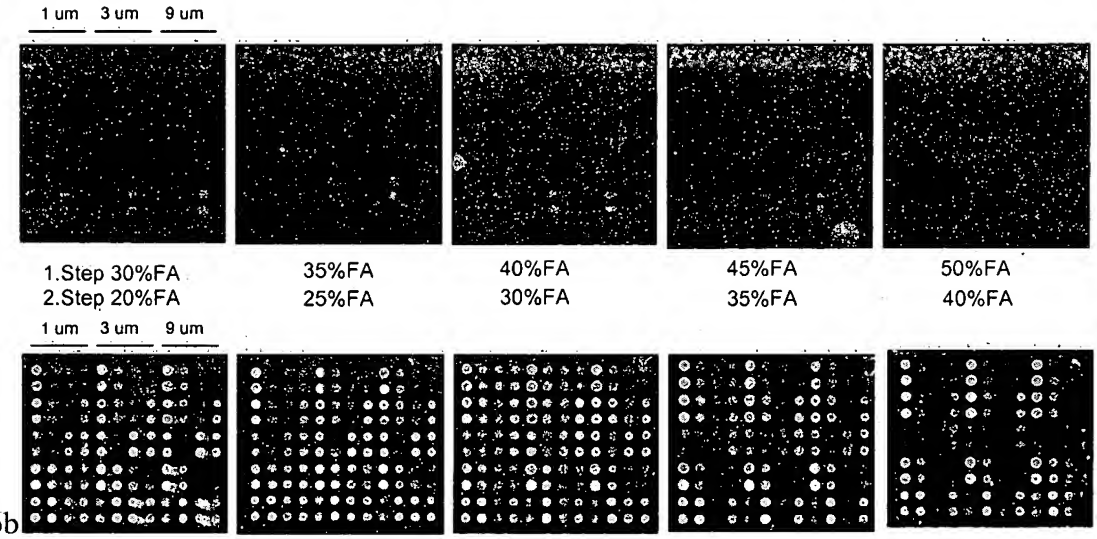


FIG.6b

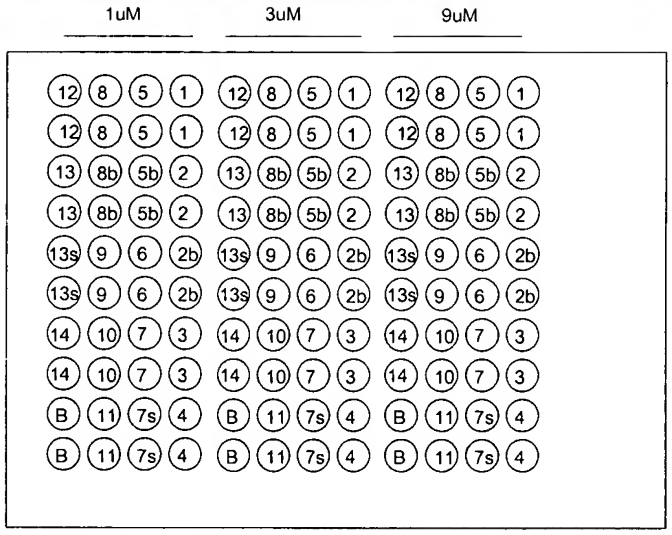


FIG. 6C

Fig. 6a shows that no probe-capture interaction on the human oligo array printed at 1uM, 3uM and 9uM of capture oligos. Experiment conditions: Capture oligos were spotted on CodeLink slides. The oligo-dT 20mer gold particle probe (1 nM) was added on to microarray in a mixture containing 20%-40% formamide, 4x SSC, 0.04% Tween, 0.02% SDS, at 40°C for 30 min. After hybridization, the arrays were washed in 0.5 M $NaNO_3$/0.02% Tween/0.01%SDS (3X) at RT, 0.5 M $NaNO_3$ (2X). The slides were subjected to silver stain (5.5 min) to obtain scatter signal. Fig. 6b shows that the high specific hybridization signal was achieved with 1 ug of total human reference RNA in a 2h hybridization. Experiment conditions: Capture oligos were spotted on CodeLink slides. 1 ug of human total reference RNA was hybridized on a microarray in a mixture containing 30%-50% formamide, 4x SSC, 0.04% Tween, 0.02% SDS, at 40°C for 2h. After hybridization, the arrays were washed in 0.5 M $NaNO_3$/0.02% Tween/0.01%SDS (3X) at RT, 0.2XSSC, 10seconds (2X), and spin dry. The arrays were further hybridized with 1nM dT 20mer-gold nanoparticle probe in a mixture containing 20%-40% formamide, 4x SSC, 0.04% Tween, 0.02% SDS, at 40°C for 30 min. The arrays were washed in 0.5 M $NaNO_3$/0.02% Tween/0.01%SDS (3X) at RT, 0.5 M $NaNO_3$ (2X). The slides were subjected to silver stain (5.5 min) to obtain scatter signal. Fig. 6c is the array layout for arrays showed in Figure 6 and Figure 7.

FIG. 7

FIG. 7a

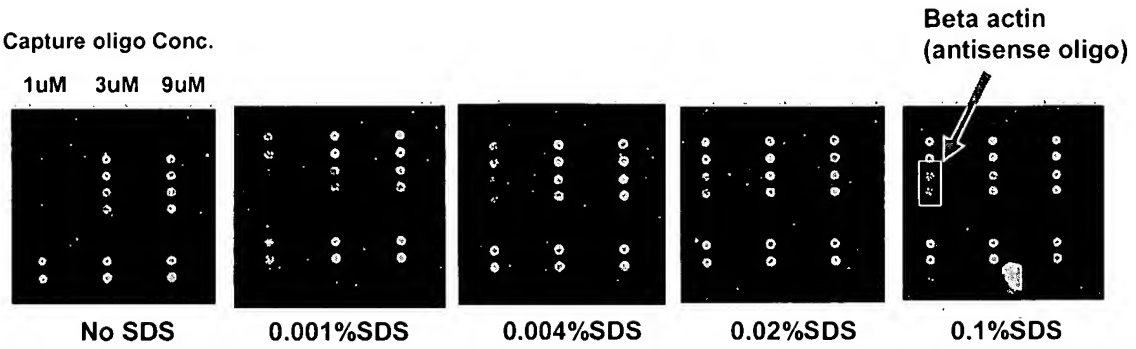


FIG. 7b

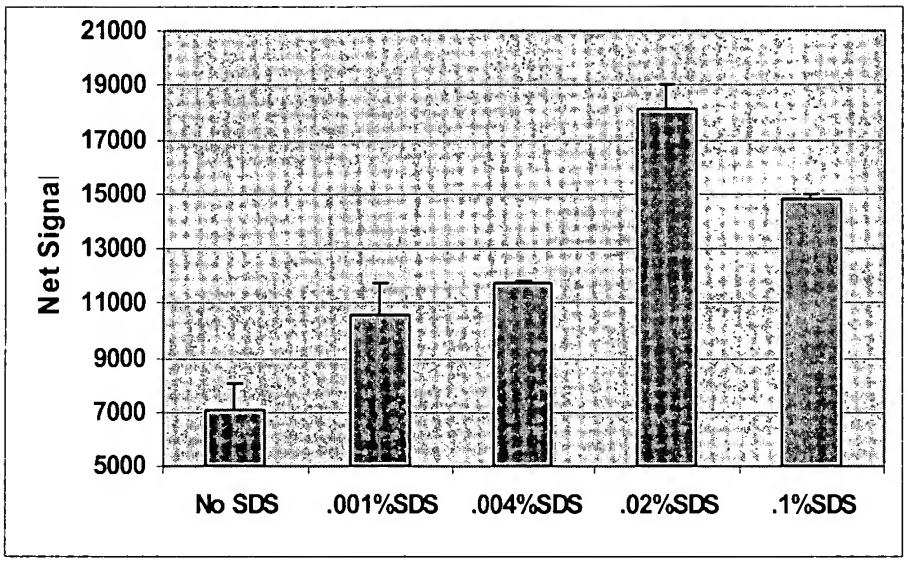


Fig. 7a shows that the combination of SDS and Tween 20 increases hybridization signal. Hybridization image with 0.1 ug of total human universal reference RNA. Experiment conditions: Capture oligos (at 1uM, 3uM and 9uM) were spotted on CodeLink slides. 0.1 ug of human total reference RNA was hybridized on a microarray in a 5ul of mixture containing 50% formamide, 4x SSC, 0.04% Tween, and different SDS concentrations as indicated, at 40°C for 1.5h. After hybridization, the arrays were washed in 0.5 M $NaNO_3$/0.02% Tween/0.001%SDS (3X) at RT, 0.2XSSC, 10seconds (2X), and spin dry. The arrays were further hybridized with 1nM of dT 20mer-gold nanoparticle probe in a mixture containing 30% formamide, 4x SSC, 0.04% Tween, and different SDS concentrations as indicated, at 40°C for 30 min. The arrays were washed in 0.5 M $NaNO_3$/0.02% Tween/0.001%SDS (3X) at RT, 0.5 M $NaNO_3$ (2X). The slides were subjected to silver stain (5.5 min) to obtain scatter signal. Fig. 7b is a plot of signal intensity of beta actin spots printed at 1uM. Experiment conditions: The net hybridization signal of beta actin spots (capture oligo at 1uM) in Fig. 7a was measured and plotted for each SDS concentration.

FIG. 8

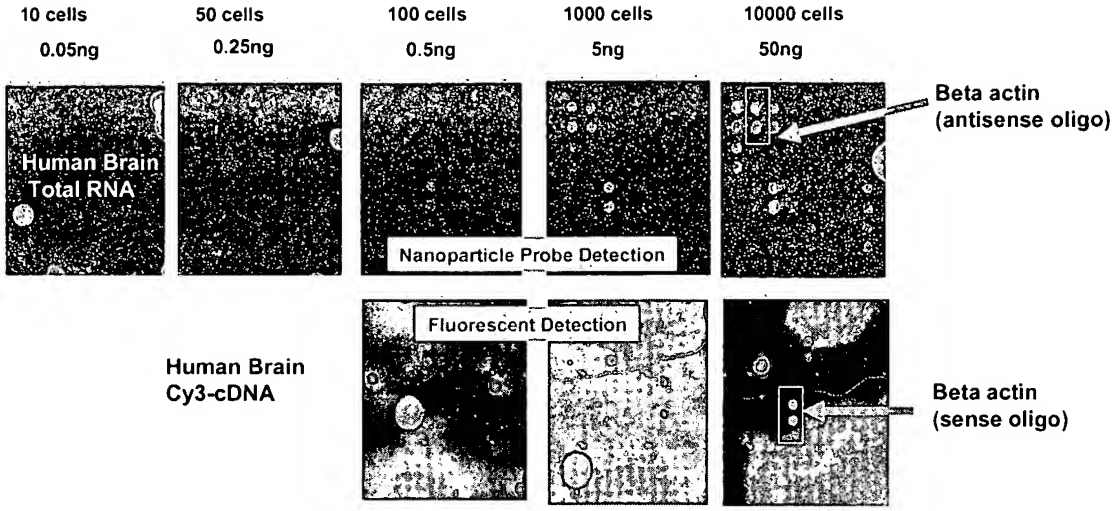


FIG. 8a

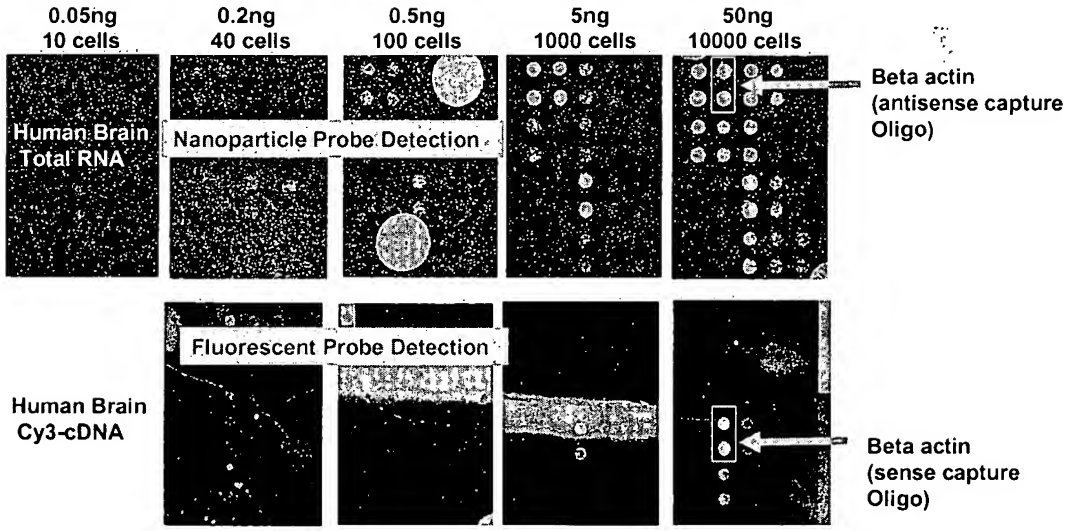


FIG. 8b

Fig. 8a is Comparison of Sensitivity with 2h Assay. Serial dilutions of human reference RNA as well as total brain RNA were made to final concentrations from 50 ng/rxn to 0.05 ng/rxn. (RNA supplier: BD Bioscience Clontech). A two-hour target hybridization was performed in the presence of 46% FM, 4X SSC /0.04% TW20, 0.01% SDS in a 5 ul reaction. Slide was washed 3 times with 0.5N $NaNO_3$/0.02%TW20/0.01% SDS and twice with 0.2X SSC followed by probe hybridization (32% FM, 4X SSC /0.04% TW20 /0.005% SDS) for 25 min. Slide was again washed 3 times with 0.5N $NaNO_3$/0.02%TW20/0.005% SDS and twice with 0.5N $NaNO_3$ followed by silver development and light scattering detection. Fig. 8b is sensitivity Comparison with 24h Hybridization. Serial dilutions of human reference RNA as well as total brain RNA were made to final concentrations from 50 ng/rxn to 0.05 ng/rxn. (RNA supplier: BD Bioscience Clontech). An overnight target hybridization was performed in the presence of 46% FM, 4X SSC /0.04% TW20, 0.01% SDS in a 5 ul reaction. Slide was washed 3 times with 0.5N $NaNO_3$/0.02%TW20/0.01% SDS and twice with 0.2X SSC followed by probe hybridization (32% FM, 4X SSC /0.04% TW20 /0.005% SDS) for 25 min.Slide was again washed 3 times with 0.5N $NaNO_3$/0.02%TW20/0.005% SDS and twice with 0.5N $NaNO_3$ followed by silver development and light scattering detection.

FIG. 9

FIG. 9a

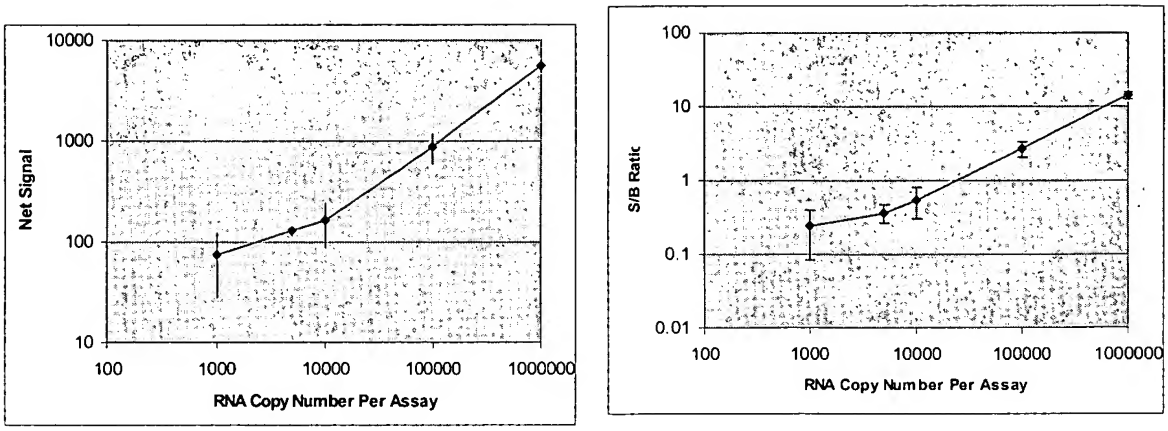
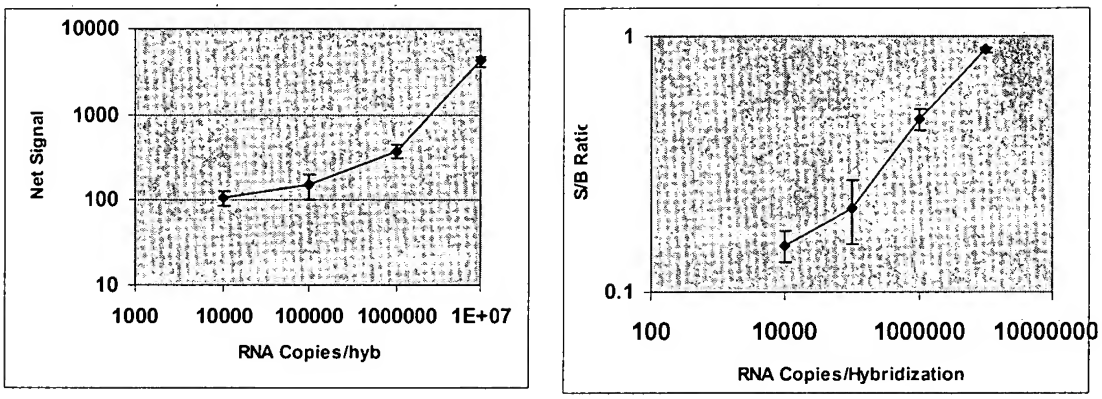


FIG. 9b



Sensitivity of RNA Detection. The net signal intensity and signal/background ratio of control gene #4 were plotted. Fig. 9a is RNA hybridization for 24 hours. Fig.9b is RNA hybridization for 2 hours. Experiment conditions: Capture oligos (control gene number 4, from Ambion) were spotted on CodeLink slides. Control RNA number 4 (in vitro transcript) titration (experiment was performed to evaluate the sensitivity of RNA detection. For 24h hybridization, the input of RNA molecules ranged from 1,000,000 per hybridization to 1,000 per hybridization. For 2h hybridization, the input of RNA molecules ranged from 10,000,000 per hybridization to 10,000 per hybridization. The RNA was hybridized on microarrays in a 5ul of mixture containing 48% formamide, 4x SSC, 0.04% Tween, and 0.01% SDS, at 40°C for 2h or 24h as indicated. After hybridization, the arrays were washed in 0.5 M $NaNO_3$/0.02% Tween/0.01%SDS (3X) at RT, 0.2XSSC, 10seconds (2X), and spin dry. The arrays were further hybridized with 1nM of dT 20mer-gold nanoparticle probe in a mixture containing 35% formamide, 4x SSC, 0.04% Tween, and 0.01% SDS, at 40°C for 30 min. The arrays were washed in 0.5 M $NaNO_3$/0.02% Tween/0.01%SDS (3X) at RT, 0.5 M $NaNO_3$ (2X). The slides were subjected to silver stain (5.5 min) to obtain scatter signal.